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EXAMINER

CANELLA, KAREN A

ART UNIT	PAPER NUMBER
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1642

DATE MAILED: 06/04/2003

10

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/760,119

Applicant(s)

Bacus et al

Examiner

Karen Can Ila

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 months MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☐ Responsive to communication(s) filed on _____.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-6 is/are pending in the application.
- 4a) Of the above, claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-6 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
a) ☐ All b) ☐ Some* c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
*See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).
a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892) 4) ☐ Interview Summary (PTO-413) Paper No(s). _____
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 5) ☐ Notice of Informal Patent Application (PTO-152)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). 4 6) ☐ Other:

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DETAILED ACTION

1. Acknowledgment is made of applicants election of the species of "apoptosis".
2. Claims 1-6 are pending and under consideration.

Claim Rejections - 35 USC § 112

3. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

4. Claims 3 and 6 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 3 is rendered vague and indefinite in the reliance on the terms p21, p27, and p16 as the only means of characterizing the biological markers upon which the method depends. The designation of "pX" is conventional in the art for a protein having a molecular weight of X. It can read on any protein of that molecular weight. Further, it is unclear whether the recitation of said biological markers encompasses only wild type proteins, or if it encompasses non-functional proteins as well. In the case of p21, the metes and bounds cannot be determined because p21 is recognized in the art to refer to the WAF1/CIP1 protein or the ras p21 protein, and the two proteins differ in structure and biological activity. In the case of p27, the metes and bounds cannot be determined because p27 is known as the p27 antigen, INF-inducible protein p27, beta4 integrin binding protein p27, and cyclin dependent kinase N1B p27 protein, all of which represent proteins of different structures and activities.

For purpose of examination, all alternatives will be considered.

The recitation of "biological sample" in claim 6 lacks proper antecedent basis in claim 5.

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Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 1, 2, 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bacus (U.S. 5,288,477) in view of the abstract of Bacus et al (Breast Cancer research and Treatment, 1999, vol. 57, page 55), Warri et al (Journal of the National cancer Institute, 1993, vol. 85, pp. 1412-1418), the abstract of Wu (Cancer Research, 1996, Vol. 16, pp. 2233-2239), the abstract of Fornier et al (Oncology, 1999, vol. 13, page 647-658) and the abstract of Lebwhol et al (Annals of Oncology, 1999, 10 suppl. 6, pp. 139-146).

Claim 1 is drawn to a method for determining a response to administration of a chemotherapeutic or chemopreventative agent comprising collecting a first tissue or cell sample from an individual before exposing the individual to the chemotherapeutic or chemopreventative agent; collecting a second tissue or cell sample from the individual after exposing the individual to the chemotherapeutic or chemopreventative agent; immunohistochemically staining the first and

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the second tissue or cell samples using a detectably labeled antibody directed against a biological marker associated with apoptosis; measuring the optical density of the stained cells of step (c) wherein the stained cells are illuminated with light having a wavelength absorbed by the stain; determining whether expression of the biological marker associated with apoptosis was increased following exposure to the chemotherapeutic or chemopreventative agent. Claim 2 embodies the method of claim 1 wherein the detectable label is a chromogen or fluorophore. Claim 5 embodies the method of claim 1 wherein the optical density of the stained cells is preformed by image analysis. Claim 6 embodies the method of claim 5 wherein the image analysis is preformed by splitting a signal comprising the optical density of the stained biological sample into a multiplicity of signals that are processed using optical filters having different absorption and transmittance properties, so that each signal is specific for one of a multiplicity of stains used to stain the cells in the biological sample.

Bacus ('477) teaches a method for determining the effectiveness of a therapeutic agent in the treatment of cancer by measurement the ability of the therapeutic agent to induce terminal differentiation wherein malignant cells of the cancer over express an oncogene product comprising obtaining from a human having cancer a biopsy comprising viable malignant cells; dividing said biopsy into a first and a second portion; treating the first portion with a compound having specific binding affinity for said oncogene product; maintaining said first and second portions in physiologically acceptable medium for an amount of time sufficient to induce maturation in the viable malignant cells of the first portion; and comparing the percentage of cells in the first portion which exhibit markers of terminal differentiation with the percentage of cells in the second portion which exhibit markers of terminal differentiation, wherein the effectiveness of treatment correlated with the degree of terminal cell differentiation, or alternatively comparing the amount of oncogene product in said first portion with the amount of a oncogene product in said second portion (claims 1 and 10). Bacus teaches that cell proliferation is yet another measure of the extent of terminal cell differentiation, and that a stabilization and reduction of cell populations as compared to

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untreated control cells indicates substantial terminal differentiation (column 11, lines 53-61). Bacus teaches that induction of a translocation of the Her-2/neu receptor from the cell surface to the cytoplasm or perinuclear region of the cell induce a terminally differentiated phenotype in the tissue or cell sample is indicative of terminal differentiation and that this induction can be expedited by means of binding by antibodies (claims 4-6 and 11-17 and column 5, lines 1-38). Bacus teaches that normal cells are devoid of Her-2/neu on the surface membrane (column 4, lines 62-68). Bacus teaches the anti-Her-2/neu antibody conjugated to a fluorescent dye and indirect methods of antibody detection such as the use of peroxidase-anti-peroxidase staining or alkaline phosphatase staining, thus fulfilling the specific embodiment of claim 2. Bacus teaches that membrane bound Her-2/neu may be quantified by digitized image analysis in conjunction with fixation and staining procedures (column 11, lines 6-25). Bacus teaches that cell sample can be stained with an anti-Her-2 antibody and an additional DNA stain and that digitization of two filtered images of the single sample, one for each specific stain allows for the summation of the optical density value for the DNA stain and the optical density value for the Her-2/neu stain (column 10, lines 20-65), thus fulfilling the specific embodiments of claim 6. Bacus does not specifically teach the binding and internalization of the Her-2/neu receptor with the induction of apoptosis in the tissue or cell sample, however, Bacus includes the stabilization and reduction of a cell population as part of the definition of terminal differentiation (column 11, lines 53-61).

Warri et al teach that methods for treating breast cancer should target the induction of apoptosis to breast cancer cells (abstract, last sentence).

The abstract of Wu teaches that apoptosis is a valuable marker for response in patients having primary or adjuvant chemotherapy for breast cancer.

The abstract of Fornier et al teaches that clinical studies are underway in the treatment of breast cancer by the combined administration of Herceptin and Taxol. The abstract identifies Herceptin as a humanized antibody directed to the Her-2/neu protein.

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The abstract of Lebwohl et al teaches that recent result indicate that the combined administration of Herceptin and doxorubicin result in a higher response rate and prolongs the time to disease progression when compared to chemotherapy alone.

Bacus et al teach that Taxol and doxorubicin affect apoptotic signaling in breast cancer cells by different mechanisms, namely, Taxol activated the p38 Map kinase cascade and doxorubicin activated the p38 jun kinase pathway. Bacus et al teach that inhibition of PI-3 resulted in the inhibition of doxorubicin induced cell cycle arrest. Bacus et al teach that Herceptin inhibits Pi-3 kinase. Bacus et al conclude that over expression of Her-2/neu or Her-3 in breast cancer patients will affect a patients response to chemotherapeutic reagents.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to evaluate a patients response to chemotherapy comprising the administration of Taxol and Herceptin or the administration of deoxyrubicin and Herceptin by means of obtaining a sample of cells or tissues from said patient before the chemotherapy and obtaining a second sample of cells or tissues from said patient after chemotherapy and quantitating the presence of Her-2/neu on the surface of said cells by means of an antibody labeled with a fluorophore or a chromogen and quantitating the total number of cells by staining DNA, and subjecting the labeled cells to image analysis wherein the image analysis is performed by splitting a signal comprising the optical density of the stained biological sample into at least two signals that are processed using optical filters having different absorption and transmittance properties so that a signal from the labeled antibody can be separated from a signal from the labeled DNA, so that a percentage of cells expressing both labeled antibody and labeled DNA can be quantified in order to measure the effectiveness of the combined therapy in the induction of apoptosis in breast cancer cells in patients having undergone therapy. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of the abstract of Lebwhol and the abstract of Fornier et al on recent clinical trials using combinations of Herceptin with Taxol and doxorubicin, the teachings of Bacus et al which calls into question the

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interaction with Herceptin on the apoptotic pathways utilized by doxorubicin and Taxol; the teachings of Warri et al on the targeting of apoptosis to breast cancer cells as a therapeutic approach for treating breast cancer, the teachings of the abstract of Wu on the correlation between the induction of apoptosis and response to chemotherapy for breast cancer, and the teachings of Bacus ('477) on the targeting of stabilization and a reduction of a cell population in a method of treating breast cancer. One of skill in the art would know that the induction of apoptosis in breast cancer cells as a result of chemotherapy would result in a stabilization and reduction of a cell population which would fall under the definition of "terminal differentiation" set forth in Bacus ('477, column 11, lines 53-61).

7. Claims 1-3, 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bacus (U.S. 5,288,477) in view of the abstract of Bacus et al (Breast Cancer research and Treatment, 1999, vol. 57, page 55), Warri et al (Journal of the National cancer Institute, 1993, vol. 85, pp. 1412-1418), the abstract of Wu (Cancer Research, 1996, Vol. 16, pp. 2233-2239), the abstract of Fornier et al (Oncology, 1999, vol. 13, page 647-658) and the abstract of Lebwhol et al (Annals of Oncology, 1999, 10 suppl. 6, pp. 139-146) as applied to claims 1, 2, 5 and 6 above, and further in view of Caffo et al (Clinical Cancer research, 1996, vol. 2, pp. 1591-1599), the abstract of el-Deiry et al (Cancer Research, 1995, Vol. 55, pp. 2910-2919) the abstract of Thor et al (Journal of the National cancer Institute, 1992, Vol. 84, pp. 845-855) and the abstract of Shetty et al (Leukemia Research, 1996, vol. 20, pp. 11-12)..

The specific embodiments of claims 1, 2, 5 and 6 and the teachings of the combination of references that render obvious said embodiments are set forth above. Warri further teach that induction of apoptosis in human breast cancer cells results in an elevated level of mRNA for TGF-beta.

Claim 3 is drawn in part to the method of claim 1 wherein the biological marker is p21 or TGF-beta.

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Neither Bacus ('477) the abstract of Bacus et al, Warri et al, the abstract of Fornier et al nor the abstract of Lebwohl teach p21 as the biological marker protein. Warri et al teach that mRNA for TGF-beta is a biological marker for apoptosis (the bottom of page 1416 to the top of page 1417, "The apoptotic changes observed in this study were connected with an elevation in the levels of both TRMP-2 and TGF-beta mRNAs"). It is reasonable to conclude that TGF-b protein was elevated as a result of the elevation of TGF-beta mRNA.

Caffo et al teach that chemotherapy or radiotherapy induces DNA damage which activates P53 function, which in turn blocks the cell cycle to allow DNA repair or apoptosis, but that this activation depends on the functional status of p53. Caffo et al teach that the way to confirm the functional status of p53 is to measure downstream effector functions such as the activation of p21 (page 1596, first paragraph under the heading of "Discussion"). Caffo et al teach that the phenotype of p21 negative/ p53 positive is indicative of a phenotype which cannot activate the apoptotic cascade in response to DNA damaging drugs (page 1591, second column, lines 1-6). Caffo et al teach that in breast cancer patients treated with systemic adjuvant therapy, p21 positive/ p53 positive tumors were associated with long disease free survival and long overall survival, but that patients having p21 negative/ p53 positive tumors had short disease free survival and short overall survival (page 1591, first column lines 17-21 and table 3, under the heading "Patients treated with any adjuvant therapy"). . Caffo et al conclude that the p21/p53 phenotype may be of clinical relevance concerning the response to chemotherapy/hormone therapy and that the p21 negative/ p53 positive phenotype could corresponds to a situation where p53 is expressed but lacks transcriptional activity because of mutational or functional inactivation and that this phenotype reflexes the complete abrogation of p53 function; Caffo et al further teach that in p21 negative/ p53 positive cases the tumor cells have an impaired G1 checkpoint and may not be able to activate the apoptotic cascade in response to DNA damaging chemotherapy and thus can be more prone to treatment failure by conventional therapy (page 1599, first full paragraph).

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The abstract of el-Deiry et al teaches that antibodies to human p21 can be used in immunohistochemical analysis to monitor the effects of radiation induced damage.

The abstract of Thor et al teaches that antibodies to human p53 can be used in immunohistochemical analysis to detect p53 in archival samples of breast carcinomas.

The abstract of Shetty et al teaches that antibodies to human TGF-beta can be used in immunohistochemical analysis to monitor the expression of TGF-beta in cells of myelodysplastic syndromes.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to include the antibodies which bind to p21, p53 and TGF-beta to the method rendered obvious by the combination of Bacus ('477) the abstract of Bacus et al, Warri et al, the abstract of Fornier et al and the abstract of Lebwhol. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Warri et al on the up regulation of expression of TGF-beta after treatment with an anti-estrogen compound which causes apoptosis in breast cancer cells; the teachings of Caffo et al on the importance of the p21 and p53 phenotypes of breast tumors in the response to chemotherapy, and the teachings of the abstracts of el-Deiry et al and Thor et al and Shetty et al who teach that antibodies to p21, p53 and TGF-beta are available and useful for immunohistochemistry. Furthermore, one of skill in the art would conclude that if the remaining tumor cells were p21 negative/ p53 positive, chemotherapy should be stopped.

8. Claims 1-3, 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bacus (U.S. 5,288,477) in view of the abstract of Bacus et al (Breast Cancer research and Treatment, 1999, vol. 57, page 55), Warri et al (Journal of the National cancer Institute, 1993, vol. 85, pp. 1412-1418), the abstract of Wu (Cancer Research, 1996, Vol. 16, pp. 2233-2239), the abstract of Fornier et al (Oncology, 1999, vol. 13, page 647-658) and the abstract of Lebwhol et al (Annals of Oncology, 1999, 10 suppl. 6, pp. 139-146) as applied to claims 1, 2, 5 and 6

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above, and further in view of Hochhauser (Anti-Cancer Drugs, 1997, vol. 8, pp. 903-910), the abstract of Ohtani et al (Cancer, 1999, vol. 85, pp. 1711-1718) and the abstract of Emig et al (British Journal of Cancer, 1998, Vol. 78, pp. 1661-1668).

The specific embodiments of claims 1, 2, 5 and 6 and the teachings of the combination of references that render obvious said embodiments are set forth above.

Claim 3 is drawn in part to the method of claim 1 wherein the biological marker is p27 or p16.

Neither Bacus (U.S. 5,288,477), the abstract of Bacus et al, Warri et al, the abstract of Fornier et al nor the abstract of Lebwhol teach p27 or p16 as the biological marker protein.

Hochhauser teaches that alterations in cell cycle genes can sensitize cells to apoptosis following treatment with chemotherapeutic agents (page 908, first sentence under the heading "Conclusion"). Hochhauser teaches that induction of p16 expression results in reversible cell cycle arrest which renders cells resistant to a variety of chemotherapeutic agents including methotrexate, cisplatin and vincristine (page 906, second column, lines 7-13). Hochhauser also teaches that expression of p27 in tumors is related to acquired drug resistance to chemotherapeutic agents (page 907, under the heading "p27 and chemosensitivity").

The abstract of Ohtani et al teaches that antibodies to human p27 can be used in immunohistochemical analysis to monitor the expression of p27 in gastric cancer cells and that decreased levels of p27 are indicative of decreased rates of apoptosis in said cells.

The abstract of Emig et al teaches antibodies to human p16 can be used in immunohistochemical analysis to monitor the expression of p16 in breast cancer cells.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to include antibodies to p16 and antibodies to p27 in the method rendered obvious by the combination of Bacus ('447), the abstract of Bacus et al, Warri et al, the abstract of Wu, the abstract of Fornier et al and the abstract of Lebwhol et al. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by

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the teachings of Hochhauser on the inverse relationship between the expression of p27 and p16 and the induction of apoptosis by chemotherapeutic agents, and the teachings of the abstracts of Ohtani et al and Emig et al on the availability of antibodies to human p16 and p27 and usefulness of said antibodies for immunohistochemistry.

9. Claims 1, 2, 4 and 5 are rejected under 35 U.S.C. 103(a) as being unpatentable over the abstract of Meyn et al (Anticancer Drugs, 1995, vol. 6, pp. 443-450) in view of Riss (U.S. 6,350,452) and Bjorklund et al (WO 99/16789) and Schlossman et al (U.S. 5,935,801) and Desjardins (U.S. 5,972,622)..

The abstract of Meyn teaches seven different murine tumors comprising a mammary adenocarcinoma, an ovarian carcinoma, a lymphoma, three sarcomas, and a squamous cell carcinomas were examined 8 and 24 hours after treatment in vivo with cisplatin or cyclophosphamide and that the mammary adenocarcinoma, ovarian carcinoma, and lymphoma exhibited significant apoptosis in response to cisplatin or cyclophosphamide. The abstract further teaches that the mammary carcinoma and the ovarian adenocarcinoma also underwent apoptosis in response to adriamycin, 5-fluorurcil, Ara-C, etoposide, camptothecin, and fludarabine. Meyn et al conclude that apoptosis is a feature of tumor response to chemotherapy in vivo and notes the heterogeneity of apoptotic response between different tumor types and to different cytotoxic agents.

Riss teaches antibodies that recognize an epitope of the PARP protein formed by cleavage of said protein by caspases (column 3, lines 26-51). Riss teaches a method for detecting apoptosis in a cell or a group of cells including tissue samples and biopsy samples (column 4, lines 1-20) comprising detecting the neo-epitope bound by the anti-PARP protein by means of ELISA, immunohistochemistry, immunocytochemistry and flow cytometry (column 4, lines 21-46). Accordingly Riss contemplates detectably labeled antibody with a fluorophore or chromogen

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(column 4, lines 47-64, and column 14, line 45 to column 16, line 40), thus fulfilling the specific embodiments of claim 2, drawn to the labeling of the antibody with a fluorophore or chromogen, claim 4, drawn to ELISA assay and claim 5 drawn to image analysis, which would be satisfied by flow cytometry..

Bjorklund et al teach a method for detecting early apoptotic changes in epithelial cells comprising contacting said cells with the M30 antibody which binds to an epitope of keratin 18 that is exposed after cleavage by caspases (page 2, lines 1-9 and 29-32). Bjorklund et al teach that the preferred embodiments of the method include determination of the rate of apoptosis, which is useful in the diagnosis of diseases such as cancer and for monitoring the effect of therapy (page 10, lines 5-8). Bjorklund et al teach that the antibody can be used in different immunoassays labeled in accordance with the actual assay used (page 10, lines 25-27) and contemplates enzymes and fluorescent markers (page 8, lines 30-31). Bjorklund et al teach the immunoassay which may be used in the method include ELISA and dissociation enhancement time-resolved fluoroimmunoassay, thus fulfilling the specific embodiments of claims 2, 4 and 5. Bjorklund et al teach that the M30 antibody staining is applicable to fresh, formalin fixed paraffin embedded tissue sections from biopsy (page 17, lines 30-34).

Schlossman et al teach an antibody which binds to an epitope localized on the membrane of mitochondria, 7A6, wherein said epitope is present only in cells undergoing apoptosis (column 1, lines 5-9, column 5, lines 38-65). Schlossman et al teach that said antibody is labeled with a fluorophore or chromogen and used in flow cytometry or ELISA assays to detect apoptotic cells (column 7, line 47 to column 8, line 44, column 9, line 59 to column 10, line 13) thus fulfilling the specific limitations of claims 2, 4 and 5. Schlossman et al teach that said antibody can be used in vitro in assays which compare the level of apoptotic cells in treated and untreated tumor cells (column 12, lines 39-43) and to monitor the efficacy of therapeutic regimens (column 2, lines 41-47).

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Desjardins teaches the need for markers of apoptosis in order to determine whether apoptosis has been induced in tumor cells by cancer chemotherapy. Desjardins teaches that identification of said markers would be an improvement over the prior art which relies on direct measurements of tumor size in vivo (column 2, lines 54-63). Desjardins teaches anti-GP46 antibodies, which specifically target apoptotic cells (column 8, lines 7-15) which can be used to monitor the treatment of a disease (column 6, lines 15-17). Desjardins teaches the labeling of said antibodies with fluorophores and chromagens (column 11, lines 3-18), and the detection of said labeled antibody in antibody-antigen complexes, by any procedure known in the art, such as ELISA and fluorescent immune assay, including single and double antibody techniques (column 11, lines 54-56). Further, any immunoassay known in the art would also include flow cytometry. Thus, Desjardins teach the specific embodiments of claims 2, 4 and 5.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to use any of the antibodies taught by Riss, Bjorklund et al, Schlossman et al or Desjardins in a method of monitoring the efficacy of chemotherapy in an individual, wherein a sample of cells or tumor tissue was taken from said individual before and after the administration of a chemotherapeutic drug, and wherein the analysis was done by means of ELISA or image analysis. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Meyn et al on apoptosis as a feature of tumor response to chemotherapy in vivo, and the heterogeneity of apoptotic response between different tumor types and to different cytotoxic agents; the teachings of Bjorklund et al on the use of the M30 antibody on monitoring the effect of therapy; the teachings of Schlossman et al on the use of the anti-7A6 antibody in monitor the efficacy of therapeutic regiments; and the teachings of Desjardins on the use of the anti-GP46 antibody in determining whether apoptosis has been induced in tumor cells by cancer chemotherapy. One of skill in the art would be motivated to substitute the assay based on antibody binding for the conventional assays of tumor size measurement because Desjardins teaches that said conventional assays require at least a month of

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treatment before a detectable difference would be measured, whereas an immunoassay on cells or tissues taken from the individual before and 8 or 24 hours after chemotherapy would measure the percentage of apoptotic cells resulting from one treatment. Thus, it would be possible to determine a likelihood of a response to a particular chemotherapeutic agent after one treatment rather than a month of treatments.

10. Claims 1, 2 and 4-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over the abstract of Meyn et al (Anticancer Drugs, 1995, vol. 6, pp. 443-450) in view of Riss (U.S. 6,350,452) and Bjorklund et al (WO 99/16789) and Schlossman et al (U.S. 5,935,801) and Desjardins (U.S. 5,972,622) as applied to claims 1, 2, 4 and 5 above, and further in view of Bacus (U.S. 5,288,477). The specific embodiments of the claims are recited above, and the prior art teachings which render obvious said embodiments. The combination of Meyn et al and Riss and Bjorklund et al and Schlossman et al and Desjardins do not specifically teach the embodiments recited in claim 6, drawn to the use of optical filters for the separation of the signals produced by a multiplicity of stains.

Bacus teaches that cell sample can be stained with an antibody and an additional DNA stain, and that digitization of two filtered images of the single sample, one for each specific stain allows for the summation of the optical density value for the DNA stain and the optical density value for the antibody stain (column 10, lines 20-65), thus teaching the specific embodiments of claim 6.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to include a DNA stain with the detectably labeled antibody and perform image analysis by splitting a signal comprising the optical density of the stained biological sample into a multiplicity of signals, comprising at least one signal for a DNA stain and one signal for an anti-apoptotic antibody stain which are processed using optical filters having different absorption and transmittance properties, so that each signal is specific for one of a multiplicity of

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stains used to stain the cells in the biological sample. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Bacus on the inclusion of a DNA stain to determine the total number of cells in the sample (column 10, lines 20-65). One of skill in the art would know that the DNA stain would serve to quantify the total number of cells and thus the ratio of the antibody stain to the DNA stain would give the percentage of apoptotic cells in a sample.

11. Claims 1, 2, 4 and 5 are rejected under 35 U.S.C. 103(a) as being unpatentable over the abstract of Meyn et al (Anticancer Drugs, 1995, vol. 6, pp. 443-450) in view of Riss (U.S. 6,350,452) and Bjorklund et al (WO 99/16789) and Schlossman et al (U.S. 5,935,801) and Desjardins (U.S. 5,972,622) as applied to claims 1, 2, 4 and 5 above, and further in view of the abstract of Booth et al (Apoptosis, 1996, Vol. 1, pp. 191-200), the abstract of Shen et al (Cancer, 1998, Vol. 82, pp. 2373-2381), the abstract of Hiraishi et al, Glycobiology, 1993, Vol. 3, pp. 381-390), the abstract of Cutrona et al (Journal of Experimental Medicine, 1995, vol. 181, pp. 699-711), the abstract of Frankfurt et al (anticancer Research, 1996, Vol. 16, pp. 1979-1988),

The abstract of Booth et al teaches that antibodies raised to the peptide DVVDADEYLIPQ were are a useful marker of apoptotic cell in the intestinal epithelium.

The abstract of Shen et al teaches that the Ki-67 antibody is indicative of apoptosis.

The abstract of Hiraishi et al teaches that antibodies which bind to Ley are indicative of apoptosis.

The abstract of Cutrona et al teaches that expression of CD10 and CD38 on the surface of lymphoma cells was indicative of said cells undergoing apoptosis.

The abstract of Frankfurt et al teaches that monoclonal antibodies which bind to single stranded DNA are indicative of apoptosis.

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It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to use any of the above antibodies in the method of detecting apoptosis as a result of chemotherapy as rendered obvious by the combination of Meyn et al, Riss, Bjorklund et al, Schlossman et al and Desjardins. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the abstracts of Booth et al, Shen et al, Hiraishi et al, Cutrona et al Frankfurt et al and Attallah et al all who all teach alternative antibodies which specifically bind to apoptotic markers.

12. Claims 1, 2 and 4 and 5 are rejected under 35 U.S.C. 103(a) as being unpatentable over the abstract of Meyn et al (Anticancer Drugs, 1995, vol. 6, pp. 443-450) in view of Riss (U.S. 6,350,452) and Bjorklund et al (WO 99/16789), Schlossman et al (U.S. 5,935,801) and Desjardins (U.S. 5,972,622) and Bacus (U.S. 5,288,477)) as applied to claims 1, 2 and 4-6 in section 10 above, and further in view of Pamukcu et al (U.S. 5,852,035), Smith-McCune et al (WO 99/24620) and the abstract of Attallah et al (Hepato-Gastroenterology, 1996, Vol. 43, pp. 1305-1312). Claims 1, 2, 4 and 5 are drawn in part to methods for determining the response of administration of a chemopreventative agent to an individual. The combination of Meyn et al, Riss, Bjorklund et al, Schlossman et al and Desjardins render obvious the specific limitations of the claimed methods with respect to determining the response to a chemotherapeutic agent. Neither Meyn et al, Riss, Bjorklund et al, Schlossman et al nor Desjardins teach a method for determining the response to a chemopreventative agent.

Pamukcu et al teach a method for treating pre-malignant lesions including colonic polyps and cervical dysplasia by administering compounds which induce apoptosis in said neoplastic tissues (column 5, lines 13-32).

Smith-McCune et al teach a methods of screening for cervical dysplasia and cervical cancer comprising the measurement of apoptotic cells in cervical samples (page 3, lines 16-25,

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page 10, lines 24-26). Smith-McCune et al teach that the apoptotic rate is unregulated in dysplastic tissue (page 8, lines 19-20).

The abstract of Attallah et al teaches that antibodies which bind to CK1 can be used to quantify apoptotic epithelial cells in premalignant lesions of the gastric mucosa.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to use the method rendered obvious by the combination of Meyn et al, Riss, Bjorklund et al, Schlossman et al and Desjardins in a method of determining a response to a chemopreventative agent, such as those compounds taught by Pamukcu et al, in an individual having pre-malignant lesions or dysplasia. One of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Pamuku et al on the induction of apoptosis in pre-malignant lesions as a target for therapy; and the teachings of Smith-McClune on the correlation between apoptotic rate and dysplasia and the teachings of the abstract of Attallah et al on the use of antibodies to quantify apoptosis in premalignant lesions.

Conclusion

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Karen Canella whose telephone number is (703) 308-8362. The examiner can normally be reached on Monday through Friday from 8:30 am to 6:00 pm. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached on (703) 308-3995. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.


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